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Growth and sporulation of *Phytophthora ramorum* in vitro in response to temperature and light

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Abstract: *Phytophthora ramorum*, recently found in the US, is causing concern for hardwood forests and the nursery industry. In an effort to identify some of the environmental limitations to growth and sporulation we undertook a laboratory study of four US and three European (EU) isolates. On V8 media, isolates grew when incubated at 2–28 C and produced chlamydospores at 8–28 C. Sporangia were produced at all temperatures tested: 10–30 C for US isolates and 6–26 C for EU isolates. Optimal temperatures were 16–26 C for growth, 14–26 C for chlamydospore production and 16–22 C for sporangia production. US isolates grew less and produced fewer spores when exposed to increasing doses of near-UV radiation (50–300 $\mu W/cm^2$) and visible radiation (250–1500 $\mu W/cm^2$). EU isolates were exposed to 300 $\mu W/cm^2$ near-UV only, which significantly reduced growth of one of three isolates and had no significant effect on spore production. In our studies *P. ramorum* tolerated a broad range of temperature and light conditions, which suggests that it is capable of establishment in a wide geographic area.

Key words: near-UV radiation, sudden oak death, visible radiation

INTRODUCTION

The oomycete fungus *Phytophthora ramorum* Werres, DeCock & Man in't Veld first was identified and described by Werres et al (2001) from isolates collected in Germany and the Netherlands from *Rhododendron* and *Viburnum* spp. Rizzo et al (2002) confirmed *P. ramorum* as the cause of sudden oak death (SOD) affecting tanoak (*Lithocarpus densiflorus*) and *Quercus* spp. in California. On the trunks of susceptible oaks the disease is characterized by the development of bleeding cankers, which often lead to mortality. Localized infestations of tanoak have been reported in southern Oregon (Goheen et al 2002) and nurseries on the West Coast have been sites of infection as well (Parke et al 2004, Davidson et al 2005, Rizzo et al 2005).

P. ramorum has a wide host range, with documented hosts in 27 families (Jones 2005). On woody plants other than oaks, infection is usually foliar and may travel down the petiole into the stem (Werres et al 2001). Popular nursery plants such as rhododendron, camellia, lilac (*Syringa*), mountain laurel (*Kalmia*), Japanese andromeda (*Pieris*) and yew (*Taxus*) are susceptible to infection by *P. ramorum* (Davidson et al 2003, Tooley et al 2004). In 2001 the Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture implemented a federal emergency order restricting the movement of nursery stock from California, Washington and Oregon in an effort to prevent the spread of *P. ramorum* from the Northwest to other areas of the country (USDA-APHIS 2002). On 22 Apr 2004, APHIS issued an amended emergency order, which implemented new restrictions on interstate movement of host nursery stock and associated articles from all commercial nurseries in California that are outside the quarantined area (USDA-APHIS 2004). On 10 Jan 2005, an emergency order added nurseries in Oregon and Washington that ship interstate and listed 84 plant species to be regulated as nursery stock. The documented host range of *P. ramorum* continues to increase as additional plant species are examined (Davidson et al 2003, Henricot and Prior 2004, Parke et al 2004, Jones 2005).

Just as with *P. lateralis*, a closely related species (Hansen et al 2000), the origins of *P. ramorum* remain unknown. The first populations examined from the two continents represented different mating types, with A1 identified from Europe and A2 from western North America (Werres et al 2001, Rizzo et al 2002). Recently however the A1 mating type also has been found in the western US. (Hansen et al 2003). Other differences have been reported between the two populations as well. For example European isolates have been reported to be more virulent and faster growing in culture than American isolates (Brasier 2003). This raises concerns about the possibility for genetic recombination should the European A1 strain become established in the US

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and undergo recombination with the A2 strain; the potential exists for increased virulence and broader host range or geographical fitness of the progeny.

Although Werres et al (2001) reported some basic biology of *P. ramorum*, the effects of temperature and light on growth and sporulation of *P. ramorum* have not been examined in detail as they have in some other *Phytophthora* species (Ribiero et al 1976, Ribiero 1983). As *P. ramorum* moves eastward in the US on infected shipments of nursery stock (Stokstad 2004) such data could provide guidance in assessing the capacity of the pathogen to become established in new areas and predicting its potential geographic range. Comprehension of the effects of temperature on *P. ramorum* growth also could influence the design and implementation of *P. ramorum* surveys, which could be modified according to temperatures occurring in different geographic regions, as well as sampling and culture protocols to promote growth and production of identifying characteristics. Further the effects of temperature and light may provide information useful in future experiments investigating host range and conditions conducive for infection and survival of the pathogen. Clarification of the temperature extremes, beyond which *P. ramorum* does not grow, might help explain why it is difficult to isolate the pathogen during certain seasons of the year. Hayden et al (2004) reported lower detection frequencies for the pathogen in California in Nov–Feb (rainy, cool) and Jul–Aug (dry, warm) each year compared with Mar–Jun (moderate moisture, temperature). Knowledge of the effects of light on sporulation might provide insight into the types of environmental conditions under which larger numbers of spores may be produced; this information could prove useful in future studies of sporulation and/or aggressiveness. Thus, in an effort to increase our understanding of the biology of *P. ramorum*, we evaluated the effect of exposure to various temperatures and light on the growth and sporulation capacity of four American and three European *P. ramorum* isolates.

MATERIALS AND METHODS

Studies on American (US) isolates were performed in an APHIS-approved laboratory at the University of Rhode Island. Origins of US isolates are: 73101 (Tidwell, from *Lithocarpus densiflorus*, Sebastopol, California); Pr52 (Rizzo, from *Rhododendron catawbiense* ''Gomer Waterer'', Felton, California); Pr5 (Rizzo, from *L. densiflorus*, Marin County, California); Pr6 (Rizzo, from *Quercus agrifolia*, Marin County, California). Studies on the European (EU) isolates were conducted in a biosafety level 3 greenhouse containment facility at Fort Detrick, Maryland. EU isolates were: BBA9/95 (Werres, *P. ramorum* type culture [CBS 101553] isolated from *R. catawbiense*); BBA11 (Werres, BBA 15/01-11, from *Viburnum* sp., Germany); BBA18 (Werres, BBA 15/01-18 from *Viburnum bodnantense*, Germany). Isolates were maintained in liquid nitrogen until use.

P. ramorum was cultured on V8 juice agar medium modified from Englander and Roth (1980): 100 mL commercial V8 juice (Campbell Soup Co., Camden, New Jersey) clarified by centrifugation (1000 *g* for 10 min) then filtered through Whatman GF/A glass microfiber paper, 0.3 g $CaCO_3$, 0.02 g β-sitosterol dissolved in 10 mL hot ethanol 95%, 17 g agar, and 890 mL deionized water. Cultures were incubated at 20–22 C unless otherwise specified. Petri dishes were sealed with Parafilm.

US Isolates.—Temperature. The temperature gradient equipment used in this study was developed by C.M. Leach (1967). Aluminum plates (60 × 122 cm) were fitted along a pair of opposite edges with copper tubing. Water from a heated reservoir was pumped continuously through the tubing on one edge of each plate and water from a refrigerated reservoir was circulated through the other. Water temperatures were controlled thermostatically. A gradient of isothermal lines of 10–35 C was established along each plate. Temperatures were monitored hourly (HOBO-H8 recorders, Onset Computer Corp., Bourne, Massachusetts) with thermistor sensors affixed along the surface of each plate. Preliminary experiments delineated approximate cardinal temperatures for the isolates, and gradient temperature extremes were set accordingly for detailed tests. Dimensions of each gradient plate allowed for the placement of six 60 mm Petri dishes at a given temperature. Temperatures on the thermal gradient plates fluctuated ca. ± 1 C from the desired temperature. A growth chamber was employed to determine growth rates and spore production by colonies at 2, 4, 6 and 8 C, temperatures beyond the capability of the gradient apparatus.

Four mm plugs were removed from the perimeter of ca. 2 wk cultures and inverted in the center of 60 mm plastic Petri dishes containing 7 mL V8 juice agar media. Three dishes from each of two isolates of *P. ramorum* were placed along isothermal lines at increments of 2 C on each of the two thermal gradient plates, for a total of six replicates/isolate/temperature. Colonies were exposed to low levels of visible and near-UV radiation (15 μW/cm² near-UV). Radial growth of colonies was measured at two perpendicular diameters after 3, 6, 9, 12 and 14 d incubation. Daily growth (mm) was determined by the equation: (colony diam at 9th d—diam inoculum plug)/9(d). After 14 d each colony was fixed with 1 mL formaldehyde/acetic acid/alcohol (FAA; 440 mL ethanol 95%, 50 mL acetic acid, 60 mL formaldehyde, 450 mL deionized water) then spore production was assessed.

Colonies first were examined microscopically for the presence of sporangia. When present, sporangia were harvested by rinsing the colony surface with a forceful stream of water from a spray bottle into another 60 × 15 mm Petri dish. The dish was placed over a transparent template on which a 5 mm grid was etched to aid in

counting, and total numbers of sporangia were counted with a stereomicroscope under dark field illumination. Dilutions were made when needed to aid counting. To evaluate chlamydospore production Petri dish contents were transferred individually to a 250 mL stainless steel homogenizing flask containing 15 mL deionized water, fitted on a Waring blender and comminuted at low speed for three 30 sec cycles. For each replication a 100 μL aliquot of the homogenate was transferred to a microscope slide placed over a 5 mm grid to aid counting and chlamydospores were counted with a stereomicroscope under bright field illumination. The number of chlamydospores produced in 14 d per cm^2 of colony was determined by the equation: total number of spores/(area of colony – area of inoculum plug).

Because sporangia production by US isolates was inconsistent on 10% V8 juice agar medium (data not shown) the effect of temperature on sporangia production was evaluated by incubating culture plugs in sterile 1% soil extract 7 d on the thermal gradient plates over the range of temperatures previously described. Soil was collected under a 30 y old Port-Orford-cedar (*Chamaecyparis lawsoniana*). Soil extract was prepared by adding 1% (wt/vol) soil to deionized water; this mixture was autoclaved, filtered and re-autoclaved. Three 4 mm plugs were removed from the perimeter of 10 d old colonies and transferred to each 60 mm diam Petri dish. Soil extract was added to cover plugs (approximately 14 mL). Six dishes per isolate were placed on the thermal gradient apparatus at 2 C increments from 10 to 30 C. Sporulating plugs were transferred to new Petri dishes daily, covered in fresh soil extract and replaced at their original positions on the thermal gradient plates. Dehisced sporangia remaining in the used soil extract were fixed by adding 0.5 mL FAA to each dish. Spore suspensions from each replicate/temperature were consolidated and a 7 d cumulative sporangia count for each replicate/temperature was made by transferring a 5 mL aliquot of spore suspension to a 60 mm dish, diluting as needed, and counting with a grid beneath the Petri dish.

Near-UV and visible light intensity. Radial growth and spore production by colonies in the dark or exposed to diverse intensities of light were evaluated. Three near-UV, blacklight blue (BLB) fluorescent tubes (range 310–420 nm, peak 355 nm) were suspended above a shelf in an incubator maintained at 22 C. We measured sites with near-UV light intensities of 50, 100, 200 and 300 μW/cm^2 with a Wescor Spectroline DSE-100× digital radiometer, and mapped these oval intensity patterns on a piece of poster board placed on the incubator shelf. Three cool white (CW) fluorescent tubes (range of less than 380 nm to greater than 700 nm, peak 430–590 nm) yielded intensities of 250, 500, 1000 and 1500 μW/cm^2 visible light as measured by a Li-Cor LI-185A Quantum/Radiometer/Photometer, with the pyranometer sensor. Lights were programmed for a 12 h diurnal cycle.

Six mm diam plugs were removed from 14 d old cultures and inverted onto the center of 60 mm dishes containing 7 mL of 10% V8 juice agar. Petri dishes were placed along each of four isoradiant intensities in replicates of three for each isolate. Additional cultures were incubated in the dark by wrapping in aluminum foil. Radial growth (mm) was measured after 3, 6, 9, 12 and 14 d incubation. After 14 d colonies were fixed with FAA and chlamydospores were harvested and counted. The experiment was performed twice and the data pooled. Daily growth rate and number of chlamydospores/cm^2 for each colony were computed as described previously.

To examine the effect of light intensity on sporangia production, three 4 mm diam V8 juice agar plugs were removed from 7 d old cultures and transferred to each 60 mm diam Petri dish. Sterile 1% soil extract was added to cover plugs, and dishes were arranged in the incubator to achieve the desired illumination. Five replications of each isolate were evaluated in separate experiments. Sporulating plugs were transferred daily to new dishes containing sterile soil extract, and dehisced sporangia left behind were fixed in FAA. Soil extract containing dehisced sporangia from each replicate/light intensity was consolidated and cumulative sporangia production over 7 d determined for each isolate as described previously.

EU isolates.—Temperature and light. EU isolates were studied similarly to US isolates, with the following differences. The thermal surface on the temperature gradient apparatus was 35 × 125 cm, and a single near-UV light intensity was tested. Two dishes of each of three isolates were placed along the gradient plate at locations providing temperatures of 6–30 C, in increments of 4 C. Half the dishes were illuminated with two BLB 40 watt fluorescent tubes (300 μW/cm^2 near-UV) on a 12 h diurnal cycle. The other half were kept dark by wrapping the top and sides of dishes with aluminum foil. The experiment was repeated three times. Radial growth and chlamydospore production were measured in the same manner and at the same incubation intervals as US isolates, however, sporangia production was assessed only on intact colonies. After incubation EU colonies were fixed by the addition of 1 mL 95% ethanol.

Statistical analyses.—Data were subjected to analysis of variance and/or linear regression with SAS (SAS Institute 1999).

RESULTS

US isolates.—Temperature. In analysis of variance, the factors isolate, temperature and their interaction were significant ($P < 0.001$) in relation to growth rate and spore production by the US isolates. Trace amounts of mycelial growth from inoculum plugs by the four American isolates were visible at the end of 14 d incubation at 2 C. No growth was observed from plugs incubated at 30 or 32 C. Mean daily growth rate of colonies incubated at different temperatures is displayed (FIG. 1A). Optimal temperatures ($P < 0.01$) for radial growth and daily growth rates were: Pr5, 16–26 C, 1.7–1.9 mm; Pr6, 20–24 C, 2.4–2.5 mm;

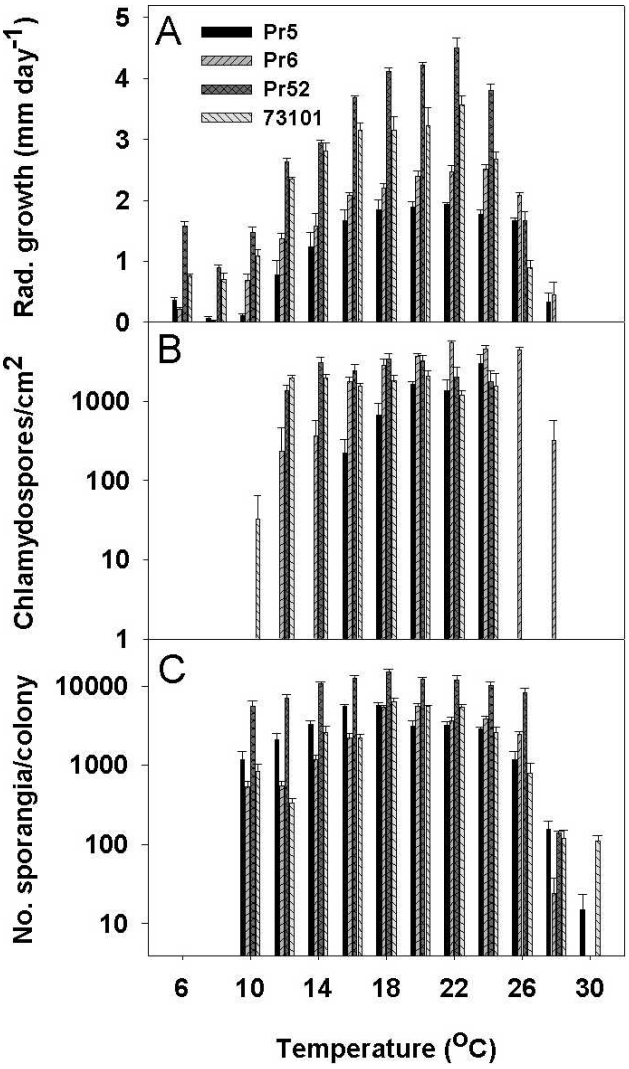

FIG. 1. Effect of temperature on mean ± SEM (A) radial growth over 9 d, (B) chlamydospore production (n = 6) over 14 d, and (C) sporangia production (n = 5) over 7 d on V8 media by US isolates of *P. ramorum*.

Pr52, 22 C, 4.5 mm; and 73101, 20–22 C, 3.2–3.6 mm.

Chlamydospores were produced in V8 media over a wide range of temperatures (10–28 C) (FIG. 1B). Optimal temperatures for chlamydospore production ($P < 0.01$) were: Pr5, 24 C; Pr6, 22–24 C; Pr 52, 14–20 C; 73101, 14–24 C. Sporangia production on V8 juice agar plugs held in soil extract also occurred over a broad range of temperatures (10–30 C) (FIG. 1C). Optimal temperatures for sporangia production ($P < 0.01$) were: isolate Pr5, 16–18 C; Pr6, 18– 20 C; Pr 52, 16–18 C; 731011, 8–22 C.

Near-UV and visible light intensity.—Negative linear relationships existed between near-UV light intensities and growth or chlamydospore production by four US isolates of *P. ramorum* tested ($P < 0.001$), with increasing light intensities resulting in diminished growth and spore production (FIG. 2A, B), apparent even at 50 μW/cm^2, the lowest intensity applied. A negative linear relationship between near-UV light intensity and sporangia production ($P < 0.001$) was apparent for three (Pr5, Pr6 and 73101) of four isolates (FIG. 2C). Sporangia production by Pr52 was highest at 100 μW/cm^2.

Visible light had a lesser effect on growth and sporulation by *P. ramorum* than did near-UV light, although a linear relationship also was apparent (FIG. 3). In general only higher light intensities (1000 or 1500 μW/cm^2) reduced growth rates and spore production (FIG. 3). As was the case with near-UV light, isolate Pr52 did not have a linear response to illumination with visible light.

EU isolates.—Temperature and light. The factors isolate and temperature, and their interaction, significantly affected radial growth ($P < 0.001$) by all three EU isolates. All isolates grew at 6 C, the lowest temperature tested, but not at 30 C (FIG. 4). Growth by the type culture, BBA9/95, was significantly slower than the other two isolates. Optimal temperatures for colony growth in V8 media were 18–22 C, with daily growth rates (mm d^{-1}) of 1.06–1.25 for BBA9/95, 3.32–3.58 for BBA11, and 3.05–3.34 for BBA18. Exposure to near-UV light significantly affected growth only by BBA9/95 ($P < 0.001$). Growth was significantly reduced by exposure to light at 6, 10, 14 and 22 C (FIG. 4).

Temperature ($P < 0.0001$) but not light significantly affected chlamydospore production. All isolates produced chlamydospores at 10–26 C, and BBA11 also at 6 C (FIG. 5A). Optimal temperatures for chlamydospore production were 22–26 C. The factors isolate ($P = 0.016$), temperature ($P < 0.001$) and their interaction ($P = 0.015$) all had a significant effect on sporangia production by EU isolates on V8 media. All isolates produced some sporangia at 6–26 C, and 22 C was found to be optimal (FIG. 5B).

DISCUSSION

We have characterized isolates of *P. ramorum* as to their growth and spore production on agar medium in response to temperature and visible and near-UV light. Vegetative growth characteristics of European isolates of *P. ramorum* in response to temperatures of 2–37 C were reported by Werres et al (2001), who found that growth occurred at 2–30 C and that the

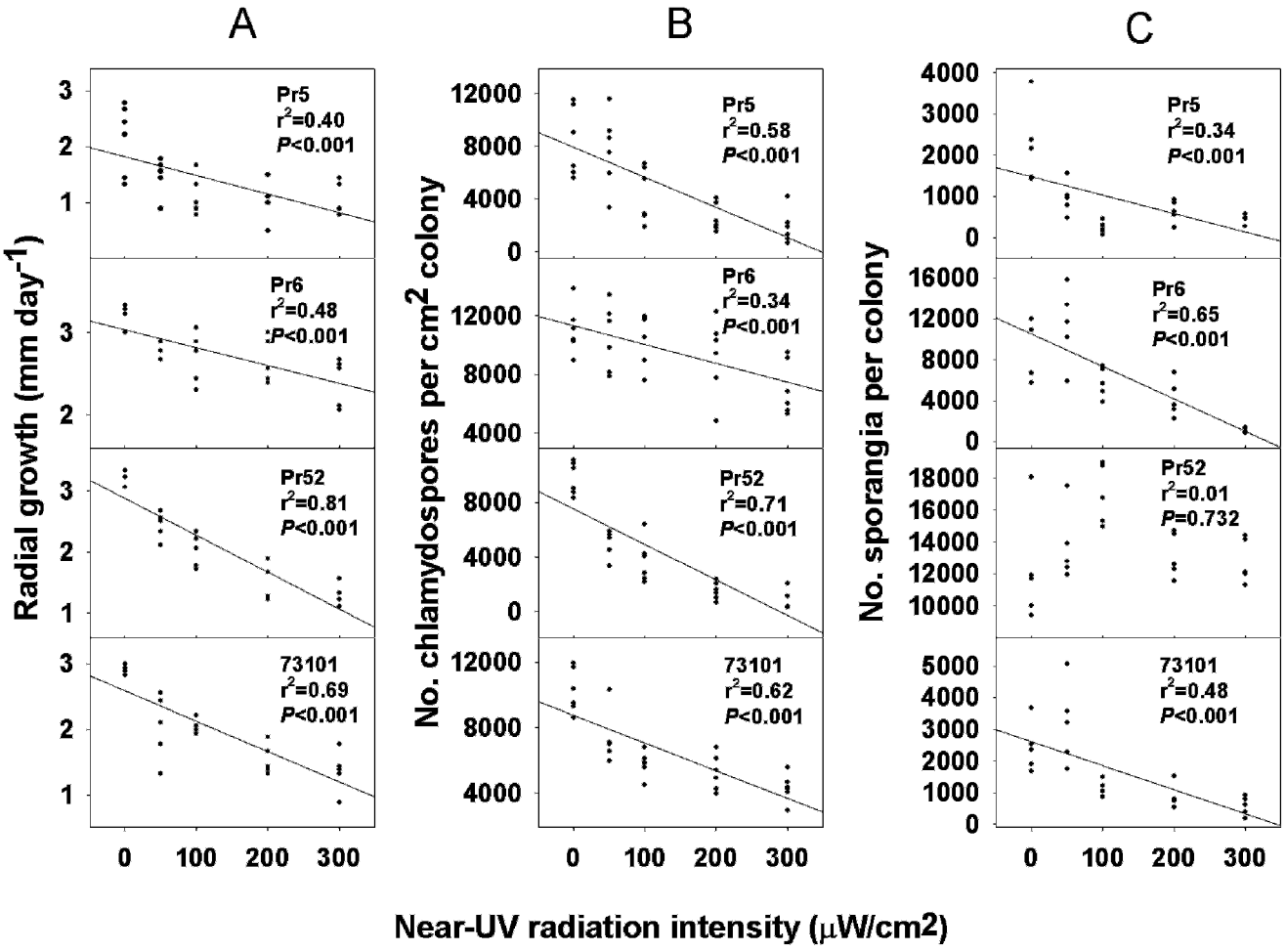

FIG. 2. Relation between near-UV light intensity and (A) radial growth over 9 d, (B) chlamydospore production (n = 6) over 14 d, and (C) sporangia production (n = 5) over 7 d on V8 media by US isolates of *P. ramorum* at 22 C.

temperature optimum for most isolates was ca. 20 C. We evaluated one of the isolates used in the work of Werres et al (isolate BBA 9/95, type culture) and found similar results (FIG. 4).

P. ramorum isolates we studied grew over a wide range of temperatures, 2–28 C for US isolates and 6 (the lowest temperature tested) to 26 C for EU isolates. We observed a broader range of temperatures optimal for growth and a more variable daily growth rate for the isolates compared with those reported by Werres et al (2001) for European isolates (2.5–3.5 mm d^{-1} at 20 C). The temperatures that support growth for *P. ramorum* are comparable to those reported for *P. infestans*, causal agent of potato late blight (Crosier 1934), as well as *P. lateralis* (Englander and Roth 1980), a species considered closely related to *P. ramorum* (Rizzo et al 2002).

Chlamydospores were produced by our US isolates at 10–28 C and optimal temperatures for chlamydospore production was 14–24 C, depending on the isolate. Our EU isolates similarly produced chlamydospores at 10–26 C, and one isolate also produced chlamydospores at 6 C. Optimal temperatures for chlamydospore production were 22–26 C for EU isolates. These results show that *P. ramorum* is capable of producing chlamydospores over a wide temperature range. In general chlamydospore production was favored by higher temperatures than were optimal for sporangia production. In comparison *P. lateralis* grows vegetatively over a broad range of temperatures yet exhibits a narrow optimum temperature range for chlamydospore production (Englander and Roth 1980). This suggests that *P. lateralis* chlamydospores might serve as a mechanism for over-summering, particularly appropriate in the Pacific Northwest, which is characterized inland by hot, dry summers. The wider range of temperatures over which *P. ramorum* produces chlamydospores might indicate that the chlamydospore plays a larger role in survival throughout several seasons of the year compared with *P. lateralis*, although the enhanced chlamydospore production at elevated temperatures by the EU

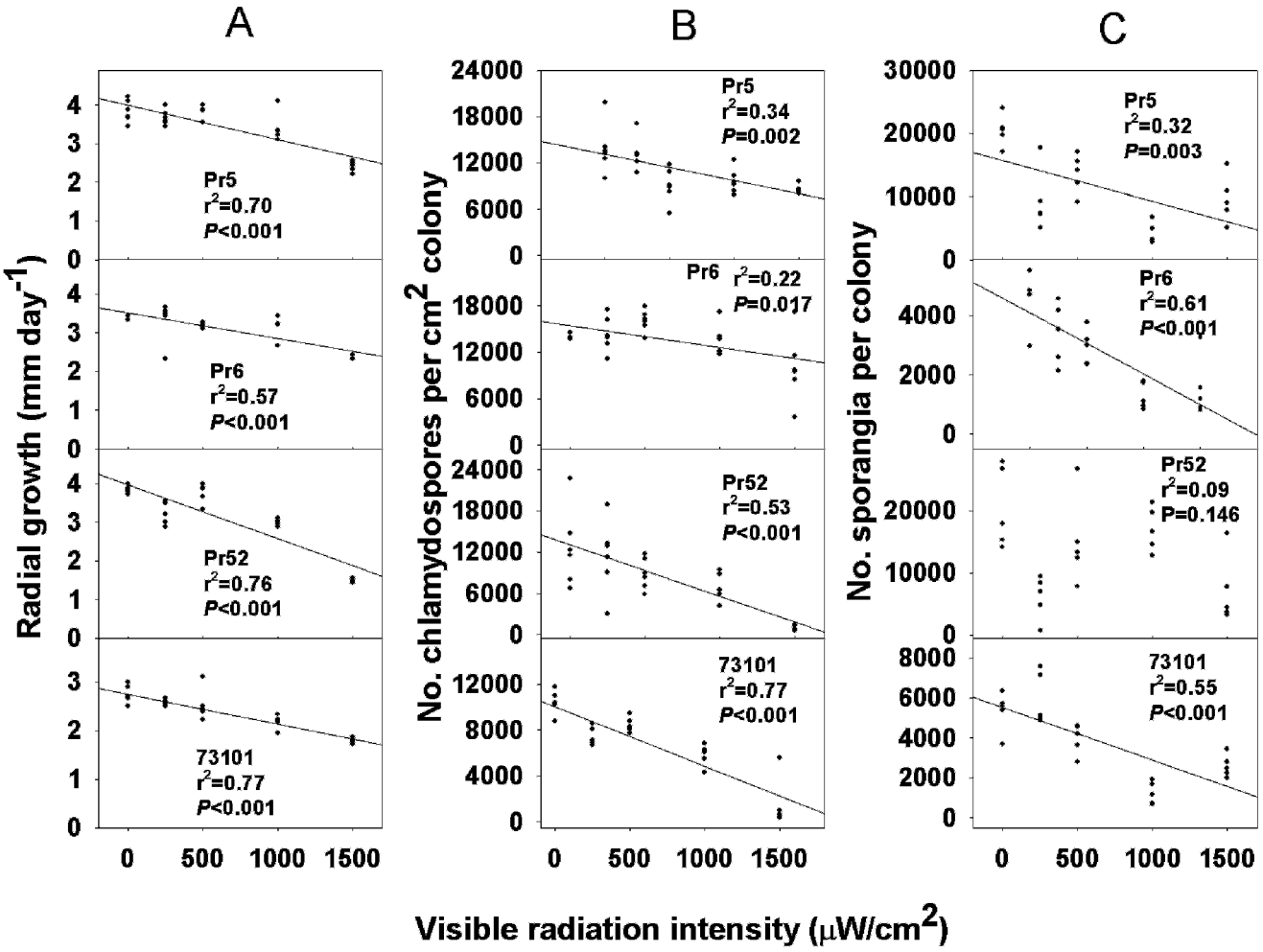

FIG. 3. Relation between visible light intensity and (A) radial growth over 9 d, (B) chlamydospore production (n = 6) over 14 d, and (C) sporangia production (n = 5) over 7 d on V8 media by US isolates of *P. ramorum* at 22 C.

isolates tested could fit the over-summering premise. Or *P. ramorum* chlamydospores may serve to let the pathogen survive times of moisture stress rather than temperature extremes.

Sporangia production by US isolates occurred over a broad range of temperatures, at 10–30 C with optimal temperature of 16–22 C, depending on the isolate. Sporangia production by two US isolates occurred at 30 C, a temperature that inhibited hyphal growth. All EU isolates studied produced some sporangia at 6–26 C, and 22 C was optimal. Our results corroborate those of Davidson et al (2005) who evaluated zoospore production from leaf disks removed from infected California bay laurel leaves and held in water at 5, 10, 15, 20, 25 and 30 C. In their first experiment the greatest zoospore production by *P. ramorum* occurred during incubation at 15 and 20 C. Results of their second experiment were less definitive. No sporangia were produced at 30 C by the isolate used in Davidson's study. For the related foliar pathogen *P. infestans*, sporangia form abundantly at 18–22 C on both tubers and leaves in saturated atmospheres, with sporulation occurring at 3–26 C but never at 1–2 C or at 27 C (Crosier 1934). *P. lateralis* produced sporangia over the broad range of 10–20 C, with the greatest numbers at 13–16 C (Englander and Roth 1980).

The breadth of the temperature optima for growth and sporulation encountered in these tests suggest that a wide range of incubation conditions would be appropriate for laboratory assays designed to isolate and identify *P. ramorum* from infected materials. Further the range of temperature optima for *P. ramorum* suggests that it easily should be capable of establishment outside the Mediterranean climate in portions of the Pacific Northwest inland where it is now prevalent in the U.S.

In contrast to the negative effect of exposure to UV light (Mizubuti et al 2000) the effects of near-UV light on fungi and oomycetes are varied and include

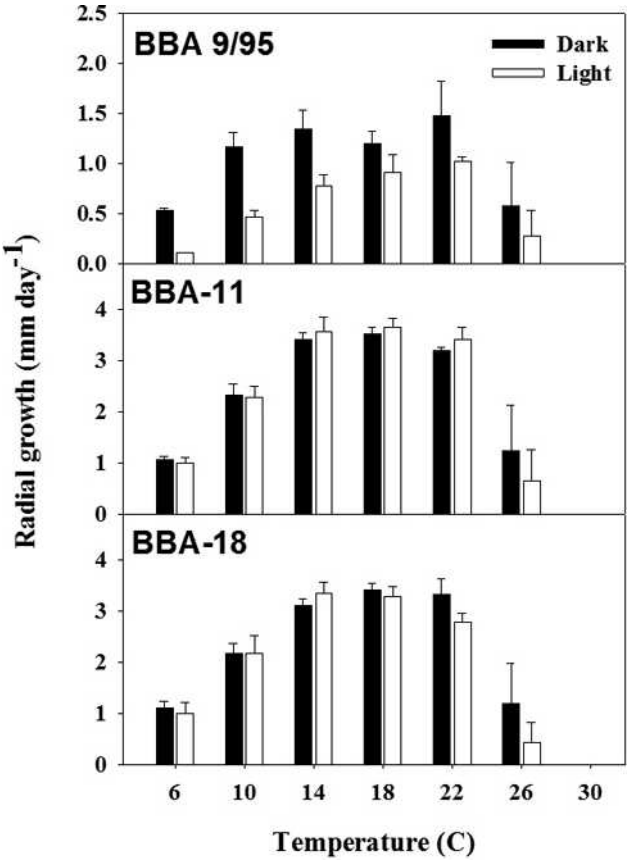

FIG. 4. Effect of temperature and near-UV radiation on mean ± SEM radial growth on V8 media by European isolates of *P. ramorum*.

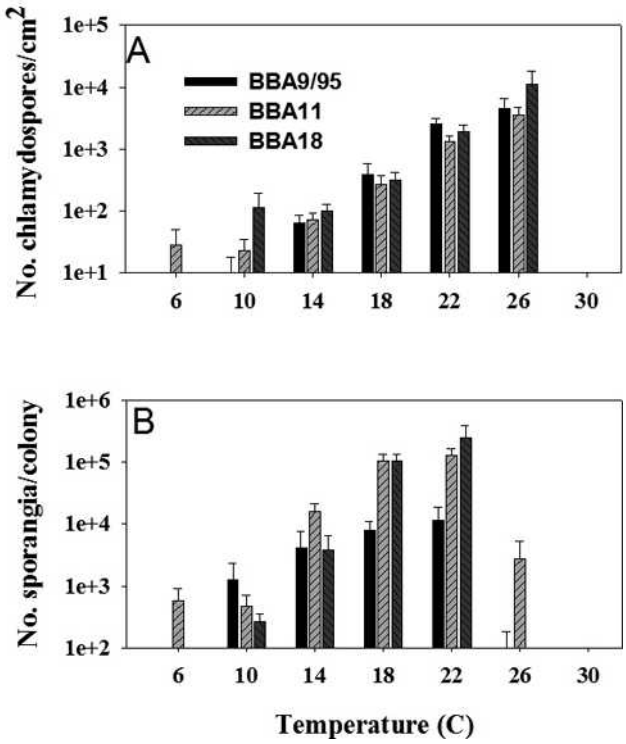

FIG. 5. Effect of temperature on mean ± SEM (A) chlamydospore and (B) sporangia production on V8 media by European isolates of *P. ramorum* in 14 d.

morphological influences, phototropism and effects on growth and sporulation (Kaiser 1973, Vakalounakis et al 1983, Harrison and Heilbronn 1988, Honda and Miyawaki 1989, Aoki and O'Donnell 1999). Reports of the effects of light on sporulation of *Phytophthora* species range from suppression, to no effect, to stimulation (Ribiero et al 1976, Ribiero 1983). In general low levels of light in the blue to near-UV range can be stimulatory in *Phytophthora* (Hendrix 1967) and sterol appears to play a role (Hendrix 1967, Englander and Turbitt 1979, Englander and Roth 1980). Enhanced sporulation in response to light by some *Phytophthoras* might be a reaction to ''unearthing'' of soilborne pathogens so that they might take advantage of a dissemination opportunity (Hansen et al 2000). In *P. cinnamomi* chlamydospore production is enhanced by near-UV light (Englander and Turbitt 1979), whereas chlamydospore production by *P. lateralis* was inhibited markedly in the presence of near-UV and cool-white fluorescent light while sporangia production was stimulated (Englander and Roth 1980).

We found that exposure to light, especially near-UV light, reduced growth and sporulation of US and one EU isolates of *P. ramorum*. Our results showed that increasing light intensities resulted in diminished growth and chlamydospore production for four US isolates of *P. ramorum*. A similar relationship was apparent for sporangia production for three of four isolates tested. Visible light had less of an effect on growth and sporulation by *P. ramorum* compared with near-UV light, although a negative linear relationship still was apparent. In general high light intensities (1000 or 1500 $\mu W/cm^2$) were required to reduce growth and spore production. Only one EU isolate had reduced growth in the presence of light compared with four US isolates. The EU population of *P. ramorum* is more variable compared with the US population (Brasier 2003) and thus one might expect to see greater variation in susceptibility to the effects of light within this population compared with the US population.

Our observations on the effects of light on *P. ramorum* contrast with those for some other fungi, for which near-UV light enhances sporulation (Kaiser 1973, Zentmeyer and Ribiero 1977, Harrison and Heilbronn 1988). As *P. ramorum* largely infects the above-ground parts of its host plants (Rizzo et al 2002), it is perplexing why exposure to light (even diurnal light) should reduce its sporulation capacity.

However light also failed to enhance sporulation of *P. syringae*, another foliar *Phytophthora* species that attacks some of the same hosts as *P. ramorum* (Harnish 1965). For *P. cinnamomi*, near-UV light stimulated chlamydospore production only when the medium was supplemented with sterol (Englander and Turbitt 1979). Despite inclusion of sterol in the medium used here for *P. ramorum* studies, near-UV radiation was found to inhibit both growth and sporulation of US isolates. Perhaps other nutritional amendments or host substrates would reveal a positive light response for growth as well as sporulation. Future studies should evaluate growth and spore production on host tissue (as did Davidson et al 2005) in addition to agar media, as done previously with *P. infestans* (Crosier 1934), taking into consideration potential variability among isolates and between mating types.

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